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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Timothy A. Springer et al.	Examiner:	Maher M Haddad
Serial No.:	09/945,265	Art Unit:	1644
Filing Date:	August 31, 2001		
For:	MODIFIED POLYPEPTIDES STABILIZED IN A DESIRED CONFORMATION AND METHODS FOR PRODUCING SAME		

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to the Mail Stop AF, Commissioner for Patents, Box 1450, Alexandria, VA on 1/20/06.

Patricia McKenney

DECLARATION UNDER RULE `132

MS AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Motomu Shimaoka , MD, PH.D,hereby declare and state as follows:

1. I am a research scientist at The CBR Institute for Biomedical Research, and I am a co-inventor and an applicant of the above-identified patent application. My curriculum vitae is attached hereto as an Exhibit.

2. I have read the Office Action of September 22, 2005, mailed by the US Patent and Trademark Office in this application ("Office Action"). I understand that paragraphs 3 and 6 of the Office Action raise certain questions regarding possible discrepancies between certain published sequences for the LFA-1 protein, and the corresponding wild type and modified amino acid sequences, as shown in SEQ ID NO:1 and SEQ ID NO:2 of the present application. In particular, the Office Action states that there are is a discrepancy involving the identity of the amino acids at positions 284, 287, 289, 294, 301 of SEQ ID NO:2 and the published sequence.

3. In my opinion, there is no real discrepancy in the sequences noted in the Office Action for the following reasons. The sequences in the present application are numbered based on the mature LFA-1 protein. In contrast, the sequences of the publications referenced in the Office Action are numbered based on the LFA-1 precursor protein. The LFA-1 precursor protein contains 25 more residues than the mature protein. These additional residues comprise the signal peptide that is removed upon maturation. Thus, the K287 and K294 amino acid positions of the present invention correspond to amino acid positions K312 and K319, respectively, of the published sequences.

4. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Date: 1/17/2006

Motomu Shimaoka (Title)

MD, PhD

CURRICULUM VITAE

Motomu Shimaoka, M.D., Ph.D.

DATE PREPARED: 12/21/05

Name :	Motomu Shimaoka
Office Address:	200 Longwood Ave., Rm 253, Boston, MA 02115
Home Address :	73 Longwood Ave. #2F, Brookline, MA 02446
email:	shimaoka@cbrinstitute.org
Place of Birth:	Nara, Japan
Visa status:	U.S. Permanent Resident

Education :

1989	M.D.	Osaka University Medical School, Osaka, Japan
1997	Ph.D. (Cell Biology)	Osaka University Graduate School, Osaka, Japan

Postdoctoral Training

1989	Clinical Fellow	Department of Anesthesiology, Osaka University Hospital, Osaka, Japan
1989-1992	Clinical Fellow	Department of Anesthesiology, Osaka Prefecture Hospital, Osaka, Japan
1992-1993	Clinical Fellow	Intensive Care Unit, Osaka University Hospital, Osaka, Japan
1993-1995	Postgraduate Fellow	Department of Anesthesiology, Osaka University Medical School, Osaka, Japan
1993-1994	Research Associate	Department of Bacterial Infections, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan
1995-1998	Research Associate	Department of Mucosal Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan
1998-2000	Research Fellow	Center for Blood Research, Harvard Medical School, Boston, MA

Licensure and Certification:

1998	Diplomat of Board of Japan Society of Anesthesiology

1991	Registered Anesthetist
1989	Japanese Medical License Registration

Academic Appointments:

1995-2001	Assistant Professor	Department of Anesthesia, Osaka University Medical School, Osaka Japan
2001-2003	Instructor	Department of Anesthesia, Harvard Medical School, Boston, MA
2003-	Assistant Professor	Department of Anesthesia, Harvard Medical School, Boston, MA

Hospital or Affiliated institution Appointments:

1995-2001	Staff Intensivist	Intensive Care Unit, Osaka University Hospital, Osaka, Japan
2000-present	Junior Investigator	The CBR Institute for Biomedical Research, Harvard Medical School, Boston, MA

Major Visiting Appointments;

1995-1998	Anesthetist	Toyonaka City Hospital, Osaka, Japan
1995-1998	Anesthetist	Tondabayashi City Hospital, Osaka, Japan
2002-	Research consultant	Respiratory Care, Mass. General Hospital, Boston, MA

Major Committee Assignments:

1995-1998	Infection Controlling	Member	Osaka University Hospital, Osaka, Japan

Professional Societies:

1995 – 1999	Japanese Society for Immunology	member
1995 – 1997	American Society for Anesthesiologists	member
1989 – 2004	Japanese Society of Intensive Care Medicine	member
1989 – present	Japanese Society of Anesthesiology	member
2004 – present	American Society of Hematology	member

Editorial Boards:

1998	Ad hoc reviewer	Clinical Infectious Disease
2002	Ad hoc reviewer	European Journal of Biochemistry
2005	Ad hoc member	NIH MOSS Study Section

Awards and Honors:

1997 Anesthesia and Intensive Care Medicine, Osaka University Medical School, Research Award

Funding Information

NIH RO1 (R01-AI063421, Shimaoka) 12/1/04-11/30/09
PI: Motomu Shimaoka
Title: Integrin LFA-1 antagonists
The major goals of this project are to develop and characterize novel antagonists specific to the active conformation of integrin LFA-1.

NIH PO1 (HL48675, Springer) 9/1/05-8/31/10
PI: Motomu Shimaoka
Title: The aberrant activation of integrins on leukocytes
This is one of five projects in NHLBI's Program Project Grant, 'Integrins and modular surface proteins in vasculature. I serve as a project leader. The aim of this project is to study the aberrant activation of integrins *in vivo* by generating novel genetically-engineered mice.

American Society of Hematology Junior Faculty Scholar Award 7/1/04-6/30/06
PI: Motomu Shimaoka
Title: Conformational regulation of integrin $\alpha_L\beta_2$ (LFA-1)
The major goals of this project are to understand the importance of the conformational regulation of integrin LFA-1 by using mutant mice that express the active conformation of LFA-1.

Regional, national, or international contributions

2005 Invited speaker, 2nd Annual Symposium in Cellular, Molecular and Clinical Research in Surgery, Boston

2005 Invited speaker, CBR Institute for Biomedical Research, Boston

2005 Invited speaker, Dept Anesthesia, Children's Hospital, Boston

2004 Invited speaker, Institute of Medical Science, the University of Tokyo, Japan

2004 Invited speaker, Department of Anesthesiology, Osaka University Medical School, Osaka, Japan

2004	Invited speaker, Gastroenterology, Mass General Hospital, Boston, MA
2004	Invited speaker, The CBR Institute for Biomedical Research, , Boston, MA
2002	Invited speaker, Dyax Corp., Cambridge, MA
2002	Invited speaker, The Center for Blood Research, Harvard Meidcal School, Boston, MA
2001	Invited speaker, Department of Mucosal Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan
2001	Invited speaker, Department of Immunology, Osaka Medical Center for Maternal and Child Health, Osaka, Japan
1997	Invited speaker, Department of Anesthesiology & Critical Care Medicine, Osaka University Medical School, Osaka, Japan
1997	Invited speaker, The Center for Blood Research, Harvard Meidcal School, Boston, MA
1997	Invited speaker, Laboratory of Immunology and Vascular Biology, Department of Pathology, Stanford University School of Medicine, Palo Alto, California.
1997	Invited speaker, Department of Anesthesiology, Osaka University Medical School, Osaka, Japan.
1997	Lecturer, The 7th World Congress of Intensive & Critical Care Medicine, Ottawa, Canada
1997	Invited speaker, The 7th World Congress of Intensive & Critical Care Medicine, Ottawa, Canada
1997	Session Chairman, The 7th World Congress of Intensive & Critical Care Medicine, Ottawa, Canada I chaired a workshop titled, "Nosocomial Pneumonia"
1996	Invited speaker, The 23rd Congress of Japanese Society for Intensive Care Medicine, Tokyo, Japan
1996	Invited speaker, The 16th Congress of Japanese Society for Clinical Anesthesia, Tokyo, Japan
1996	Lecturer, The 5th Meeting of Association for Prevention of Stomatological Infection, Kobe, Japan
1995	Lecturer, The 42nd Congress for Japanese Society of Anesthesiologists, Hamamatsu, Japan
1995-1998	Lecturer, Department of Anesthesiology & Critical Care Medicine, Osaka University Medical School, Osaka, Japan

1993 Lecturer, Japanese Society for Microbiology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

Publications:

Original

1) **Shimaoka, M.**, Yoh, M., Segawa, A., Takarada, Y., Yamamoto, K., and Honda, T. Development of enzyme-labeled oligonucleotide probe for detection of mecA gene in methicillin-resistant Staphylococcus aureus. J. Clin. Microbiol. 1994. 32: 1866-1869.

2) **Shimaoka, M.**, Iida, T., Ohara, A., Taenaka, N., Mashimo, T., Honda, T., and Yoshiya, I. NOC, a nitric-oxide-releasing compound, induces dose dependent apoptosis in macrophages. Biochem. Biophys. Res. Commun. 1995. 209: 519-526.

3) **Shimaoka, M.**, Yoh, M., Takarada, Y., Yamamoto, K., and Honda, T. Detection of the gene for toxic shock syndrome toxin 1 in Staphylococcus aureus using enzyme-labeled oligonucleotide probes. J. Med. Microbiol. 1996. 44: 215-218.

4) **Shimaoka, M.**, Iida, T., Ohara, A., Taenaka, N., Mashimo, T., Honda ,T., and Yoshiya, I. Ketamine inhibits nitric oxide production in activated macrophages. Br. J. Anesthesia 1996. 77: 238-242.

5) Okuda, Y., Sakoda, S., **Shimaoka, M.**, and Yanagihara, T. Nitric oxide induces apoptosis in mouse splenic T lymphocytes. Immunol. Lett. 1996. 52: 135-138.

6) **Shimaoka, M.**, Ikeda, M., Iida, T., Taenaka, N., Yoshiya, I., and Honda, T. Fucoidin, a potent inhibitor of leukocyte rolling, prevents neutrophil influx into phorbol-ester-induced inflammatory sites in rabbit lungs. Am. J. Respir. Crit. Care Med. 1996. 153: 307-311.

7) Sugimoto, M., **Shimaoka, M.**, Hagihira, S., Fujino, Y., Nishimura, S., Kamata, S., Taenaka, N., and Yoshiya, I. Tension pneumopericardium following tracheoplasty for congenital tracheal stenosis. Anaesth. Intens. Care 1997. 25: 539-541.

8) Yamada, K., **Shimaoka, M.**, Nagayama, K., Hiroi, T., Kiyono, H., and Honda, T. Bacterial invasion induced interleukin-7 receptor expression in colonic epithelial cell line, T84. Eur. J. Immunol 1997. 27: 3456-3460.

9) **Shimaoka, M.**, Fujino, Y., Taenaka, N., Hiroi, T., Kiyono, H., and Yoshiya, I. High frequency oscillatory ventilation attenuates the activation of alveolar macrophage and neutrophil in lung injury. Critical Care (Lond) 1998. 2: 35-39.

10) Sugimoto, M., **Shimaoka, M.**, Hosotsubo, K., Tanigami, H., Taenaka, N., Kiyono, H., and Yoshiya, I. Upregulation of Fas ligand mRNA expression in peripheral blood mononuclear cells after major surgery. Clin Exp Immunol 1998. 112: 120-125.

11) Egawa, T., **Shimaoka, M.**, Shimizu, N., Hagihira, S., Fujino, Y., Nishimura, S., Taenaka, N., Yoshimime, T., Yoshiya, I. A case of multiple aneurysms of the vein of Galen with heart failure due to persistent fetal circulation. J. Anesthesia 1998 12: 100-102.

12) Izumi, R,. **Shimaoka, M.**, Nagaoka, C., Komaki, M., Mizutani, A., Yoh, M., Honda, T., Taenaka, N., Yoshiya, I. Effectiveness of hand-disinfection by a flow water system using electrolytic products of sodium chloride, compared with a conventional method using alcoholic solution in a intensive care unit (letter). Critical Care (Lond) 1998 2: 79-80.

13) Nishimura, S., Fujino, Y., **Shimaoka, M.**, Hagihira, S., Taenaka, N., Yoshiya, I. Circadian secretion pattern of melatonin after major surgery. J. Pineal. Res 1998 25: 73-77.

14) Iijima, H., Takahashi, I., Hiroi, T., **Shimaoka, M.**, Kawano S., Nagano, K., Hori, M., Kiyono, H. Orally administered cholera toxin prevents murine intestinal T cell from staphylococcal enterotoxin B-induced anergy. Gastoenterology 1998, 115: 1197-1204.

15) **Shimaoka, M.**, Hosotsubo, K., Sugimoto, M., Sakaue, G., Taenaka, N., Yoshiya, I., Kiyono, H. Influence of surgucal stress on T cells: enhancement of early phase of lymphocytes activation. Anesth Analg 1998 87: 1431-1435.

16) Sugimoto, M., **Shimaoka, M.**, Taenaka, N., Kiyono, H., and Yoshiya, I. Satellate ganglion block attenuates lymphocyte activation. Reg Anesth Pain Med 1999 24: 30-35.

17) Shimazu, T., **Shimaoka, M.**, Sugimoto, H., Taenaka, N., Hasegawa, T., & the Osaka HUS Critical Care Study Group. Does blood type B protect against haemolytic uraemic syndrome? an analysis of the 1996 Sakai outbreak of *Escherichia coli* O157:H7 (VTEC O157) infection. J Infect 2000 41: 45-49.

18) **Shimaoka, M.**, Shifman, J.M., Jing, H., Takagi, J., Mayo, S.L., Springer, T.A. Computational design of an integrin I domain stabilized in the open high affinity conformation. Nature Struct. Biol. 2000. 7: 674-678.

19) Imanaka, H., **Shimaoka, M.**, Matsuura, N., Nishimura, M., Ohta, N., Taenaka, N., Kiyono, H. Ventilator-induced lung injury is associated with neutrophil infiltration, macrophage activation and TGF-β mRNA upregulation in rat lung. Anesth. Analg. 2001. 92: 428-436.

20) Lu, C., **Shimaoka, M.**, Ferzly, M., Oxvig, C., Takagi, J., Springer, T.A. An isolated, surface-expressed I domain of the integrin αLβ2 is sufficient for strong adhesive function when locked in the open conformation with a disulfide bond. Proc. Natl. Acad. Sci. U.S.A. 2001 98:2387-2392

21) Lu, C., **Shimaoka, M.**, Zhang, Q., Takagi, J., Springer, T.A. Locking in alternative conformations of the integrin αLβ2 I domain with disulfide bonds reveals functional relationships among integrin domains. Proc. Natl. Acad. Sci. U.S.A. 2001 98:2393-2398

22) **Shimaoka, M.**, Lu, C., Palframan, R., von Andrian, U., Takagi, J., Springer, T.A. Reversibly locking a protein fold in an active conformation with a disulfide bond: integrin αL I-domains with high affinity and antagonist activity in vivo. Proc. Natl. Acad. Sci. U.S.A. 2001 98:6009-6014.

23) Jun, C.D., **Shimaoka, M.**, Carman C.V., Takagi, J., Springer T.A. Dimerization and the effectiveness of ICAM-1 in mediating LFA-1-dependent adhesion. *Proc. Natl. Acad. Sci. U.S.A.* 2001 98: 6830-6835.

24) Ohta, N., **Shimaoka, M.**, Imanaka, H., Nishimura, M., Taenaka, N., Kiyono, H. Yoshiya, I. Glucocorticoid suppresses neutrophil activation in ventilator-induced lung injury. *Crit. Care Med.* 2001 29:1012-1016.

25) Sakaue, G., **Shimaoka, M.**, Fukuoka, T., Hiroi, T., Inoue, T., Hashimoto, N., Sakaguchi, T., Sawa, Y., Morishita, R., Kiyono, H., Noguchi, K., Mashimo, T. NF-kB decoy

suppresses cytokine expression and thermal hyperalgesia in a rat neuropathic pain model. *Neuroreport.* 2001. 12:2079-2084.

26) Jun, C.D., Carman, C.V., Redick, S.D., **Shimaoka, M.**, Erickson, H.P., Springer, T.A. Ultrastructure and function of dimeric, soluble intercellular adhesion molecule-1 (ICAM-1). *J. Biol. Chem.* 2001. 276:29019-29027.

27) Ma, Q., **Shimaoka, M.**, Lu, C., Jing, H., Carman, C.V., Springer, T.A. Activation induced conformational changes in the I-domain region of LFA-1. *J Biol Chem* 2002. 277: 10638-10641.

28) Takeuchi, M., Goddon, S., Dolhnikoff, M., **Shimaoka, M.**, Hess, D., Amato M.B.P., Kacmarek, R.M. Set positive end-expiratory pressure during protective ventilation affects lung injury. *Anesthesiology* 2002. 97: 682-92.

29) Salas, A., **Shimaoka. M.**, Carman, C.V., Chen S., Springer T.A. Transition from rolling to firm adhesion is regulated by the conformation of the I domain of the integrin LFA-1. *J. Biol Chem* 2002. 277: 50255-50262.

30) **Shimaoka, M.**, Lu, C., Salas, A., Xiao, T., Takagi, J., Springer T.A. Stabilizing the αM inserted domain in alternative conformations with a range of engineered disulfide bonds. *Proc. Natl. Acad. Sci. U.S.A.* 2002. 99: 16737-16741.

31) **Shimaoka, M.**, Xiao,T., Liu,JH, Yang Y., Dong,Y., Jun,CD., McCormack, A., Zhang,R., Joachimiak,A., Takagi,J., Wang,JH., Springer, TA. Structures of the αL I domain and its complex with ICAM-1 reveal a shape-shifting pathway for integrin regulation. *Cell.* 2003. 112: 99-111.

32) Vorup-Jensen, T., Ostermeier, C., **Shimaoka, M.**, Hommel, U., Springer, T.A. Structure and allosteric regulation of the αXβ2 integrin I domain. *Proc. Natl. Acad. Sci. U.S.A.* 2003 100: 1873-1878.

33) **Shimaoka, M*.**, Salas, A., Yang, W., Weitz-Schmitdt, G., Springer, TA*. Small molecule integrin antagonists that bind to the β2 subunit I-like domain and activate signals in one direction and block them in the other. *Immunity* 2003 19:391-402. (*Corresponding)

34) Sedeek, K.A., Takeuchi, M., Suchodolski, K., Vargas, S.O., **Shimaoka, M.**, Schnitzer, J.J., Kacmarek, R.M. Open-lung protective ventilation with pressure control ventilation, high-frequency oscillation, and intratracheal pulmonary ventilation results in similar gas exchange, hemodynamics, and lung mechanics. *Anesthesiology* 2003. 99: 1102-1111.

35) Yang, W., **Shimaoka, M.**, Chen JF., Springer, TA. Activation of integrin β–subunit I-like domains by one-turn C-terminal α-helix deletions. *Proc. Natl. Acad. Sci. U.S.A.* 2004. 101: 2333-2338.

36) Yang, W., **Shimaoka, M.**, Salas, A., Takagi, J., Springer, TA. Intersubunit signal transmission in integrins by a receptor-like interaction with a pull spring. *Proc. Natl. Acad. Sci. U.S.A.* 2004. 101: 2906-2911.

37) Salas A, **Shimaoka M**, Kogan AN, Harwood C, von Andrian UH, Springer TA. 2004. Rolling adhesion through an extended conformation of integrin αLβ2 and relation to α I and β I-like domain interaction. *Immunity* 2004. 20: 393-406.

38) Lu, C., **Shimaoka, M.**, Sales, A., Springer, TA. The mechanism of action and binding sites for competitive, antagonistic, allosteric and agonistic antibodies to the I domain of integrin LFA-1. *J Immunol.* 173: 3972-8. 2004.

39) Xie, C., **Shimaoka, M.**, Xiao, T., Schwab, P., Klickstein, L.B., Springer, T.A., The integrin a subunit leg extends at a Ca2+-dependent epitope in the thigh/genu interface upon activation. *Proc. Natl. Acad. Sci. U.S.A.* 101: 15422-7. 2004.

40) Vorup-Jensen, T., Carman, C.V., **Shimaoka, M.**, Schuck, P., Svitel, J., Springer, T.A. Exposure of acidic residues as a danger signal for recognition of fibrinogen and other macromolecules by integrin $\alpha_X\beta_2$. *Proc. Natl. Acad. Sci. U.S.A.* 2005. 102:1614-9.

41) Song, G., Yang, Y., Liu, J-h., Casasnovas, J.M., **Shimaoka, M.**, Springer, T.A., Wang, J-h. Structural basis of ICAM recognition revealed in a complex between the binding domains of ICAM-3 and integrin $\alpha_L\beta_2$ at 1.65 Å. *Proc. Natl. Acad. Sci. U.S.A.* 102: 3366-71, 2005.

42) Zhang, M., Alicot, EM., Chiu, I., Li, J., Verna, N., Vorup-Jensen, T., Kessler, B., **Shimaoka, M.,** Chan, R., Friend, D., Mahmood, U., Weissleder, R., Moore, FD., Carroll, MC. Identification of the target self-antigens in reperfusion injury. J. Exp. Med. 2006 (in press)

43) Song, G., Lazar, GA., Kortemme, T., **Shimaoka, M.**, Desjarlais, JR., Baker, D., Springer, TA. Rational design of ICAM-1 variants for antagonizing integrin LFA-1-dependent adhesion. J. Biol. Chem. 2006 (in press)

Reviews

1) **Shimaoka, M.**, Takagi, J., Springer, T.A. Conformational regulation of integrin structure and function. *Annu. Rev. Biophys. Biomol. Struct.* 2002. 31: 485-516.

2) **Shimaoka, M.**, Springer, T.A. Therapeutic antagonists and the conformational regulation of integrin structure and function. *Nature Rev Drug Discover* 2003. 2: 703-716.

3) **Shimaoka, M.**, Springer, T.A. Therapeutic antagonists and the conformational regulation of β2 integrin. *Curr Topic Med Chem* 2004. 4: 1485-1495.

Educational Materials

Course syllabus

1) **Shimaoka, M.** Integrins-cell adhesion molecules, In: Diamandopoulos, GT, organizer. An advanced biomedical science program course PA514.0, Harvard Medical School; 2001. p221-224.